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Abstract: Amylin is a peptide hormone that is mainly known to be produced by pancreatic -cells in response to a meal but amylin is also produced by brain cells in discrete brain areas albeit in a lesser amount. Amylin receptor (AMY) is composed of the calcitonin core-receptor (CTR) and one of the 3 receptor activity modifying protein (RAMP), thus forming AMY1-3; RAMP enhances amylin binding properties to the CTR. However, amylin receptor agonist such as salmon calcitonin is able to bind CTR alone. Peripheral amylin's main binding site is located in the area postrema (AP) which then propagate the signal to the nucleus of the solitary tract and lateral parabrachial nucleus (LPBN) and it is then transmitted to the forebrain areas such as central amygdala and bed nucleus of the stria terminalis. Amylin's activation of these different brain areas mediates eating and other metabolic pathways controlling energy expenditure and glucose homeostasis. Peripheral amylin can also bind in the arcuate nucleus of the hypothalamus where it acts independently of the AP to activate POMC and NPY neurons. Amylin activation of NPY neurons has been shown to be transmitted to LPBN neurons to act on eating while amylin POMC signaling affects energy expenditure and locomotor activity. While a large amount of experiments have already been conducted, future studies will have to further investigate how amylin is taken up by forebrain areas and deepen our understanding of amylin action on peripheral metabolism.

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Review

Amylin brain circuitry

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ABSTRACT

Amylin is a peptide hormone that is mainly known to be produced by pancreatic β-cells in response to a meal but amylin is also produced by brain cells in discrete brain areas albeit in a lesser amount. Amylin receptor (AMY) is composed of the calcitonin core-receptor (CTR) and one of the 3 receptor activity modifying protein (RAMP), thus forming AMY1 – 3; RAMP enhances amylin binding properties to the CTR. However, amylin receptor agonist such as salmon calcitonin is able to bind CTR alone. Peripheral amylin's main binding site is located in the area postrema (AP) which then propagate the signal to the nucleus of the solitary tract and lateral parabrachial nucleus (LPBN) and it is then transmitted to the forebrain areas such as central amygdala and bed nucleus of the stria terminalis. Amylin's activation of these different brain areas mediates eating and other metabolic pathways controlling energy expenditure and glucose homeostasis. Peripheral amylin can also bind in the arcuate nucleus of the hypothalamus where it acts independently of the AP to activate POMC and NPY neurons. Amylin activation of NPY neurons has been shown to be transmitted to LPBN neurons to act on eating while amylin POMC signaling affects energy expenditure and locomotor activity. While a large amount of experiments have already been conducted, future studies will have to further investigate how amylin is taken up by forebrain areas and deepen our understanding of amylin action on peripheral metabolism.

Amylin was discovered more than 30 years ago in type 2 diabetic patients and diabetic cats and was shown to aggregate in pancreatic islets [1]. Amylin is best known to be co-secreted with insulin by pancreatic β-cells in response to a meal and has been first identified as a satiating peptide hormone controlling food intake by decreasing meal size ([2] for review see [3–5]). Amylin belongs to the calcitonin peptide family which includes adrenomedullin, calcitonin and calcitonin-gene-related peptide (CGRP). The amylin peptide sequence is very well conserved across species [6] and the difference in amino acid sequence between humans and cats versus rodents prevents rodents amylin to aggregate [7]. Thus most of the amylin analogues such as pramlintide and future amylin receptor agonists in clinical development present an amino-acid sequence close to the rodent amylin. In addition to its anorectic property, amylin acts on multiple forebrain and hindbrain brain pathways to control different aspects of food intake, energy metabolism and reward. A specific chapter will explore the fact that amylin is also produced in the brain; whether and how it is secreted and regulated is still under investigation [8,9]. Last, amylin functions on gastric emptying and glucose control will be examined.

1. Amylin receptor and signaling

1.1. Amylin receptor subunits and binding

The amylin receptor (AMY) is composed of multi-subunits of the calcitonin receptor (CTR) which comprise the isoform 1A and 1B and the receptor activity modifying protein (RAMP) 1, 2 or 3 leading to the formation of AMY1, AMY2 and AMY3 [10].

CTR belongs to the family of the cell surface G coupled protein receptors. CTR presents different allosteric binding sites allowing the receptor to modulate its affinity to different ligands [10]. CTR receptors are located throughout the body. CTR is present in osteoclasts where it is the target for calcitonin but also in osteocytes, kidneys, testes, placenta, lungs and the brain. In the adult rodent brain, CTR1A and 1B have been localized using immunohistochemistry [7,11,12] and in situ hybridization [13,14]. CTR1A and 1B differ by the absence (1A) or the presence (1B) of a 16 amino acid insert in the first intracellular loop [15] leading to a poorer internalization and altered coupling to G-coupled proteins of CTR1B [16]. In the brain, CTR expression is the

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most abundant in the nucleus accumbens (NAc), ventral tegmental area (VTA) [17], arcuate nucleus (ARC), ventromedial hypothalamus (VMN) [14], lateral hypothalamus (LH), lateral substantia nigra, bed nucleus of the stria terminalis (BNST), locus coeruleus, area postrema (AP) and nucleus of the solitary tract (NTS) [12]. While most of these studies have been performed on rat brain, it is possible that slight differences might occur between rat and mouse brain [12,18].

At the protein level, RAMP location is more elusive due to the absence of specific antibodies. Nevertheless, mRNA expression of RAMP1 has been detected in the lungs, muscle and brain. RAMP2 mRNA expression was also detected in lungs, heart, skeletal muscle, endothelial tissue and brain. RAMP3 is mostly expressed in the brain and to a lesser amount in peripheral tissues such as the gut, lung, heart and kidney [19]. More specifically, in the brain, using in situ hybridization, RAMP1 is localized in the brainstem, AP, NAc, cortex, hippocampus, caudate putamen, olfactory tubercles, subcomissural organ [20]. RAMP2 mRNA expression is abundant in the hypothalamus [19] and is also expressed but at a lower level in olfactory bulb, hippocampus, choroid plexus of the 3rd, 4th and lateral ventricles and blood vessels throughout the brain [20,21]. RAMP3 is the most abundant in the dorsal thalamus [19], lateral geniculate, and lower amounts are detected in the subfornical organ (SFO) and AP. In the medio-basal hypothalamus, all three RAMPs have been detected in microglial, astrocyte and neuronal primary cell culture of male rats [22]. While the study of RAMP1 or RAMP3 is possible using global KO mouse models [23–25]. RAMP2 KO in mice is lethal due to the presence of RAMP2 in endothelial tissues and lack of RAMP2 during development leads to embryonic lethality [26].

AMY formation results from the co-localization of CTR and RAMP within the same cell. RAMP heterodimerization with CTR increases the accessibility of the CTR extracellular binding domain to enhance the binding of amylin [27]. Thus, at the cell level in rat, RAMP1, 2 and 3 and CTR1A have all been detected in the same cell in the AP with a preference for CTR being co-localized with RAMP3 [28]. Our recently published data also co-localized RAMP3 or RAMP1 mRNA and CTR peptide in the ARC and NTS [18]. It is however unknown if multiple AMY isoforms are present simultaneously in one cell and if these AMY isoforms can be specifically generated according to metabolic demands. ^{125}I amylin binding experiments on brain sections [14,29,30] or in vivo fluorescent amylin uptake [31] have uncovered amylin site-specific areas. The most prominent areas are located in the medial preoptic area (MPO), ARC, VMN, dorsomedial hypothalamic nucleus (DMN), NAc and in the hindbrain with the AP, caudal NTS and dorsal raphe [14,29,30]. Whole brain 3D imaging showed that fluorescent salmon calcitonin (sCT; amylin receptor agonist) and rat amylin distributed mainly into the ARC, AP and organum vasculosum of the lamina terminalis (OVLT) of the mouse brain. Surprisingly, no sCT signal was observed in NTS [31]. Using monkey brain sections, further experiments compared the binding of ^{125}I amylin and ^{125}I sCT. Interestingly [32,33] some areas with strong sCT binding did not bind amylin. Similarly, fluorescent amylin uptake showed signal using whole-brain imaging in the median eminence (ME) while fluorescent sCT did not [31]. These data are of importance given the use of sCT as an amylin receptor agonist which could lead to the activation of pathways that are not activated by native amylin [34]. One caveat using ^{125}I ligand binding experiment is that it only indicates whether the receptor is present but cannot indicate whether the native peptide can bind and reach these areas in a physiological setting. Thus, the uptake of fluorescent peptide and the use of whole-brain imaging may be a better physiological indicator of how a native peptide acts in the brain.

1.2. Receptor ligand affinity

Together CTR and RAMP can bind multiple ligands with different affinity [34,35]. CTR alone presents the greatest affinity for calcitonin and its receptor agonist sCT. AMY1 binds sCT, amylin and CGRP with the same affinity. Indeed, AMY1 is considered as the second CGRP receptor [33,36]. Since CGRP is a neuropeptide widely distributed in the brain and since CGRP plays an important role in controlling energy metabolism [33,36–39], it may act as a confounding factor when assessing amylin's effects particularly in the LPBN or ARC where both amylin and CGRP have binding sites [13,14,40]. AMY2 and AMY3 preferentially bind amylin and sCT compared to CGRP. The multiple allosteric binding sites present on AMY allow each ligand to preferentially bind to specific receptor conformations in order to activate downstream signaling pathways [35].

In addition to CTR, RAMP can bind another receptor, the calcitonin like receptor (CLR). CLR and RAMP1 form the primary CGRP receptor [10]. CLR-RAMP2 forms the adrenomedullin 1 receptor (AM1) and CLR-RAMP3 forms the AM2. AM1 preferentially binds adrenomedullin while AM2 binds adrenomedullin and adrenomedullin 2/intermedin with the same affinity. Thus, whole-body RAMP KO animal models assess diverse metabolic functions and not just amylin related signaling [24].

1.3. Amylin intracellular signaling

CTR is a GPCR. Thus, once the ligand is bound to the AMY, it activates several intracellular signaling pathways [34]. Amylin receptor signaling has been mostly studied using cell culture and transfected AMY receptors where amylin has been shown to activate G-coupled pathways and the production of cAMP [41]. On the other hand, it has also been shown in rats that amylin increases intracellular cGMP, and the local administration of a cGMP analog in the AP produced similar effects on eating as amylin [42,43]. In vivo, exogenous amylin activates ERK signaling [44] and induces the phosphorylation of ERK1/2 in the AP and ARC [24,44]. Under specific conditions, inhibition of ERK signaling pathway in the AP is able to reduce ERK phosphorylation and eating in rats [44]. The absence of RAMP1 and 3 reduces amylin-induced ERK phosphorylation in the ARC [22].

2. Amylin brain uptake

In 1998, Banks et al. performed a study assessing amylin's ability to cross the blood brain barrier by measuring the regional distribution within the brain of radioactively labelled amylin and insulin [45]. Using multiple-time regression analysis, the unidirectional influx constant (Ki) and volume distribution (Vi) were evaluated in pons-medulla, cerebellum, midbrain, hypothalamus, thalamus, hippocampus, striatum, frontal cortex, parietal cortex, and occipital cortex [46]. Pons-medulla had the highest levels and whereas the thalamus presented the slowest amylin uptake. The parietal cortex, occipital cortex, and the hypothalamus had uptake values that varied less than 10 % from that of the whole brain. Given the small variation between different brain areas, this study may suggest that amylin is diffused or transported into the brain but does not inform about the physiological or the pharmacological relevance of this event since amylin uptake takes place in brain areas where amylin receptor expression has not been shown [32] such as the parietal and occipital cortex.

More recently, a study compared amylin concentrations in the cerebrospinal fluid (CSF) between patients suffering from Alzheimer's disease (AD) and healthy control subjects [47]. Amylin concentrations in the CSF were not correlated to AD, however CSF amylin increased along with plasma amylin, suggesting that amylin in the CSF mostly originates from peripheral and not central amylin. It is thus important to investigate whether amylin passively diffuses into CSF or is actively transported. This can be tested by increasing peripheral amylin concentration and measuring CSF amylin levels. If CSF amylin levels stopped reflecting peripheral amylin, this would indicate saturation and thus the existence of a transporter.

The most recent study about amylin brain uptake by Zakariassen et al. described amylin and sCT brain distribution using whole brain 3D imaging and confocal microscopy following the peripheral injection of

fluorescently labelled peptides in mice. In order to investigate binding and internalization of fluorescently labelled amylin and sCT in ARC to NPY and/or POMC neurons, hrGFP-NPY and POMC-Cre:ERT2:tdTomato mice were used. NPY neurons seemed to be the preferential target for sCT since no binding was observed on POMC neurons. Whole brain imaging showed amylin and sCT distribution in AP, OVLT and ARC [31]. Moreover, amylin and sCT signals were observed in brain tissues and in the choroid plexus indicating presence of fluorescently labelled peptide in the blood vessels. Interestingly, unlike sCT, an amylin signal was observed in ME. This difference in brain distribution could be suggestive of some kind of transport mechanism behind amylin uptake. Considering the location of amylin uptake in the ME and in the medio-basal ARC, it is suggestive of tanycytic involvement in the transport similar to what has been shown with leptin [48]. The discovery of an amylin-specific transporter in brain areas with a functional blood brain barrier may suggest a physiological function for amylin uptake and that peripheral amylin can activate signaling pathways in different brain areas independently of the AP. On the other hand, it is also known that peripheral peptides can passively diffuse in the brain through periventricular fenestrated capillaries and they are quickly destroyed by peptidases present in the inter-neuronal space [49]. This may be the case with amylin uptake, considering the necessity of extremely high dose and very short time window of its detection [31]. The limited ability of peripheral amylin to reach brain regions where amylin receptors are located [32], may perhaps have been evolutionary compensated by central amylin production. More experiments are however needed to fully understand and clarify amylin brain uptake and identify how amylin can cross into the ME.

3. Amylin brain production

To this day, only a few studies have actively investigated amylin production in the brain even though first reports about the possibility of central amylin synthesis had already been published years ago [50–52]. The first paper that investigated in detail this issue, was published in 2009 by Dobolyi and they showed a 24-fold increase in amylin mRNA expression in rostral MPO of lactating rat dams compared to pup deprived dams and nulliparous females. Interestingly, no amylin mRNA expression was detected in male rats [9]. The difference in amylin expression between lactating rat dams and pup-deprived lactating rat dams may be indicative of oxytocin playing major role in inducing amylin expression since oxytocin is released in response to suckling [53]. Overall, the elevation of amylin expression in the MPO, the major brain area mediating maternal behaviors [54,55], is suggestive of central amylin's involvement in maternal adaptations. However, detailed studies will be necessary to test whether MPO amylin regulates metabolic or behavioral maternal adaptations.

The second study describing amylin expression in the brain was performed more recently by Li et al. in 2015 [8], which primarily investigated the role of prodynorphin (Pdyn) neurons in obesity. Using immunohistochemistry, they showed that Pdyn was co-expressed with amylin in multiple hypothalamic nuclei including cell bodies in MPO, ARC, LHA and fibers in paraventricular nucleus of the hypothamus (PVN), dorsal medial hypothalamus (DMH) and in the preoptic area. Amylin positive cells were mostly co-expressed with Pdyn in the LH and MPO and with orexin in the LH. Given the shape of the amylin positive cells in immunohistochemical staining and the fact that these cells were co-expressed with markers mostly found in neurons, amylin was suggested to be expressed in neuronal cells but further immunostainings are required to confirm the exact cell type of amylin positive cells. While in the study by Dobolyi, amylin mRNA expression was detected in female rats only, Li et al. observed a sexual dimorphism in mice with total hypothalamic amylin mRNA expression being significantly higher in WT female mice compared to WT male mice. However, this dimorphism was reversed in animals exposed to a 60 % HFD, suggesting that HFD increases hypothalamic amylin production in males and decreases it in females [8].

Interestingly, amylin expression was reduced in the hypothalamus of leptin-deficient ob/ob female and male mice and it was restored by 3-month treatment with exogenous leptin [8]. Neurons in the LH were also sensitive to amylin and leptin when assessed using patch-clamping [8]. Moreover, ob/ob mice that re-express leptin after tetracycline treatment [56] expressed significantly higher levels of hypothalamic amylin in both male and female mice. Since amylin has been shown to enhance leptin signaling in the medio-basal hypothalamus [57–59], these findings may lead to a conclusion that brain leptin signaling may contribute to the regulation of brain amylin expression; however, the physiological relevance of these phenomena remains to be investigated.

To investigate the role of central amylin on food intake, Li et al. infused the amylin antagonist AC187 into the lateral ventricle prior to an intraperitoneal (i.p.) leptin injection. They observed that leptin's anorexic effect in the first 2 h was abolished in groups treated with AC187. Although this result confirms previous studies about peripheral amylin's synergistic and additive effect on leptin [57,60,61], it is too early to conclude on the exact underlying mechanisms.

Together these studies confirm the secretion of amylin by discrete brain areas and its regulation seems to be controlled by sex, leptin and diet but whether brain-secreted amylin plays an important role compared to peripheral amylin remains to be assessed.

4. Amylin brain signaling and peripheral action

4.1. Hindbrain pathways: amylin regulation of food intake

Building up on a large number of studies, peripheral amylin has been shown to activate neurons in the AP which then propagate its signal to the lateral parabrachial nucleus (LPBN) and to subsequent downstream nuclei such as the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST).

4.2. The area postrema

The AP in the hindbrain, lies on the dorsal surface of the medulla oblongata at the caudal end of the fourth ventricle [62]. Lacking a blood-brain barrier, the blood hormones/peptides can move through the porous BBB in the AP and bind to neurons [63]. Furthermore, the AP is surrounded laterally and innervated by the nucleus of the solitary tract (NTS) [64], which comprises the primary sensory areas for both gustatory (facial, glossopharyngeal, and vagus nerves) and visceral (vagus nerve) afferents [65–67]. The vagus nerve also innervates the AP directly [68,69]. Thus, the AP is in a likely position to integrate chemical signals from the blood stream and receives neural signals from gustatory and visceral receptors to modulate intake and control body weight [70]. A large number of studies performed in different laboratories indicate that the AP plays a primary role in mediating effects of peripheral amylin (for review see [34]). Amylin directly activates AP neurons via the activation of AMYs, which are highly expressed in the AP [29], determining an increase of the marker of neural activation c-Fos. Indirect activation of the AP via afferent signaling (vagal or non-vagal) does not seem to be involved in amylin's effects on eating [71–77] (see section 6 for a further discussion on the role of the vagus in amylin's effects). Electrophysiological and immunohistochemical studies supported a direct action of amylin on AP neurons [42,44,78,79]. The best-studied amylin action is to reduce food-intake [34] and the primary role of AP was confirmed by the fact that AP/NTS lesions completely blocked amylin's anorectic effect [71]. Furthermore, local amylin administration into the AP recapitulates peripheral amylin's effect, whereas the amylin antagonist AC187 has the opposite effect [44]. One of the further actions of amylin is to reduce gastric emptying (see section 6), and the AP also seems to be necessary for amylin to mediate this function [73,80–82].

The phenotype of amylin and sCT activated neurons in the AP is

mainly noradrenergic; following systemic amylin administration, half of c-Fos positive neurons are co-localized with dopamine-β-hydroxylase (DBH), the key enzyme in noradrenaline (NA) synthesis and marker of noradrenergic neurons [83]. DBH-positive neurons in the AP seem to be necessary for peripheral amylin to reduce eating [84]. Previous work of our group showed that the selective ablation of the NA-containing neurons with the immunotoxin anti-DBH-saporin (DSAP) in AP is sufficient to abolish the hypophagic effect of amylin [84]. Further studies are however needed to investigate the role of the NA system in other reported amylin effects that are presumed to be mediated via the AP, such as the inhibition of gastric emptying, pancreatic glucagon secretion, the increase of energy expenditure and the modulation of CCK-induced anorexia [84]. While more than approximately half of amylin activated neurons are noradrenergic, further studies are needed to characterize the neurochemical nature of the remaining non-noradrenergic amylin activated AP neurons and their possible function. A recent electrophysiological study suggested that glutamatergic neurotransmission in the AP may mediate or rather modulate amylin's effect; indeed, AMYs appear to be mainly located on presynaptic glutamatergic terminals connecting to AP neurons, and amylin has been shown to increase glutamate release and cause cell firing [85]. In addition, vesicular glutamate transporters (VGLUT2) boutons were shown to be apposed to amylin activated noradrenergic neurons in AP [83].

A large number of studies indicate that the AP has a key role in the mediation of nausea/emesis [86–88]. Electrophysiological and immunohistochemical studies have reported that AP neurons respond to emetic agents and the ablation of AP prevents vomiting in response to emetic drugs [89]. However, such effects do not seem to be triggered by amylin. Many studies have shown that systemic amylin and sCT, or 4th ventricle amylin administration do not cause aversive or sickness-like behaviors indicative of a nausea/emesis, thus suggesting that the anorectic effects of amylin are not mediated by malaise [90–92]. Conversely, high doses of sCT (150 ug/kg) which are approx. 10 times higher than doses used routinely to study amylin's effect on eating, were recently shown to induce a conditioned taste avoidance (CTA) response [93]; thus, further studies are needed to characterize if it is a dose dependent effect. It is possible to define that emetic and amylin-anorectic signals, while acting on the same brain area, use different neural circuitry. Indeed, amylin and lithium chloride, an aversive stimuli, do no activate the same AP neurons and lithium chloride-induced c-Fos positive neurons are negative for CTR [94] but whether this is also true for NTS remains to be determined. These studies are crucial given the development of dual AMY/CTR agonists by pharmaceuticals industries to combat obesity.

4.3. The lateral parabrachial nucleus

Amylin's satiation effect is mainly mediated by the primary activation of neurons in the AP, which then signal other downstream nuclei, such as the LPBN [71,78,95]. The LPBN consists of a complex of neurons in the dorsolateral pons and it plays an important role in mediating a variety of visceral functions such as taste, respiration, central cardiovascular control and sleep [96]. The parabrachial nucleus in rodents [97] as well as primates [98] is a key relay for visceral signals from the caudal hindbrain to forebrain areas associated with appetite control. LPBN receives axonal inputs from different brain areas, such as the AP and NTS, and its neurons project to numerous forebrain regions, including the CeA and BNST [99]. The LPBN has been demonstrated to be a crucial brain site mediating the anorectic effect of amylin [37,95] because an ablation of the LPBN reduced amylin's anorectic effect and abolished the neural activation in CeA, reflected by the lack of the c-Fos expression [95]. While the phenotype of amylin activated neurons in AP has been shown to be mainly noradrenergic, the LPBN-specific neuronal populations responsible for the mediation of amylin's effect remain unknown. Previous studies showed that a high percentage of LPBN neurons express CGRP [100]. More recent studies showed that CGRP neurons inhibit feeding when activated by satiation signals such as cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and that these neurons directly project to CeA [37]. More specifically, the chemogenetic inhibition of CGRP^{LPBN} neurons partially decreased amylin's ability to reduce food-intake and these neurons where co-localized with c-Fos after systemic amylin administration [37]. Interestingly, these neurons are not just involved in mediating anorexia and satiation, but also relay a wide variety of aversive signals (food poisoning, pain, foot shock, itch etc.) and taste memories [101]. However, the exact neurotransmitter of CGRP^{LPBN} neurons that mediates amylin's effect remains unknown and is in need of more investigation. A recent study proposed that part of sCT's anorectic effect is mediated by the activation of non-CGRP^{LPBN} neurons, through the direct activation of the CTR neurons in the NTS [93]. Hence, more work is necessary to define in detail the role of LPBN neuronal populations in the mediation of amylin's effects.

Last, we can also hypothesize that amylin may activate AP and NTS neurons in parallel, and that these neurons may in turn activate different neuronal populations of the LPBN. Indeed, a large proportion of noradrenergic neurons from the AP are known to project to NTS [102] but direct catecholaminergic projections from the AP to the LPBN have also been shown to convey hormonal and enteroceptive signals from the hindbrain to the forebrain [103]. The specific role of noradrenergic AP-LPBN and AP-NTS-LPBN projections mediating amylin's effects needs to be clarified.

4.4. The central amygdala

Retrograde labelling techniques showed that a large portion of LPBN neurons innervate the CeA [104]. Neurons of the CeA are involved in emotional processing, learning, memory as well as aversive situations such as stress or fear and pain [105–107]. Less is known about CeA's role in the control of feeding behavior [108]. However, the neuroanatomical location and the phenotype of amygdala neurons suggest a potential contribution to feeding control. Reciprocal connections between the amygdala and nearly all key energy balance regulation nuclei exist and include projections to and from the hypothalamus, NTS, ventral tegmental area (VTA), NAc and parabrachial nucleus [109]. Moreover, amygdala neurons may directly sense gut hormones, as receptors for ghrelin and glucagon-like peptide 1 (GLP-1) have been identified on amygdala neurons [110,111]. Interestingly AMY receptor components are expressed in CeA [29]. Thus, the amygdala is in a key neuroanatomical position that allows the sampling and the integration of inputs relevant to feeding and metabolic status from both the internal and external milieus but whether peripheral amylin can access this brain site has never been demonstrated. A recent study showed that dopamine transmission in the amygdala controls food intake and reward; food-intake is associated with increased dopamine turnover in the amygdala and activation of dopaminergic 2 receptors (D2) reduced food-intake [112].

As mentioned, the CeA may be involved in amylin's effect on eating because systemic amylin administration induces neural activation in CeA [78] and because the ablation of LPBN reduces amylin anorectic effect and abolishes the neural activation in CeA [95]. Furthermore, CGRP^{LPBN} neurons directly project to CeA [39]. The lateral subdivision of CeA contains a subpopulation of GABAergic neurons, the PKC-δ^{+} neurons, identified by the presence of protein kinase C-δ [113]. The optogenetic activation of these neurons strongly suppresses food intake [113]. PKC-δ+ neurons in mice are activated by CCK and LiCl, typical anorexigenic signals, and the activity of these neurons is required for the inhibition of feeding mediated by these signals. They receive presynaptic inputs from anatomically distributed neurons activated by different anorexigenic agents [113]. These data suggest that CeA PKC-δ + neurons constitute an important node that mediates the influence of multiple anorexigenic signals but whether these neurons also receive LPBN mediated- amylin signaling remains to be determined. Thus,

further studies are needed to define the phenotype of amylin activated neurons in CeA.

4.5. Ventral tegmental area: amylin's role on the dopaminergic system and reward

The VTA receives projections from the AP/NTS and projects to the NAc [4,114]; recent findings have shown that the VTA and the mesolimbic reward system seem to mediate amylin's effect on energy balance [17,115]. It is thought that early satiation signals like amylin impede food consumption by moderating reward system activity, reducing its value [91,116,117]. Dopamine transmission may provide one mechanism that bridges the sensations of hunger and satiation with motivated behavior and there is evidence that peptides that govern food intake can also exert their effects on feeding via dopaminergic mechanisms [118,119]. Peripheral sCT is able to reduce VTA-stimulated release of dopamine in the NAc of rats, supporting the hypothesis that the mesolimbic system is involved in amylin's actions on food motivation and intake [120]. Further, there is also evidence for the modulation of the dopaminergic system by amylinergic action in other sites of brain such as the VTA and the lateral dorsal tegmental nucleus (LDTg) [17,121–123]. The relationship between the effects mediated by the VTA, LDTg and the AP is unclear at present. Given that LDTg is reciprocally connected to both the AP/NTS and VTA, amylinergic activation may involve a AP/NTS-LDTg-VTA pathway to modulate dopamine transmission. Currently the available data do not allow a clear distinction between a direct activation of the pertinent receptors by peripheral amylin or sCT or indirectly mediated effects [4,120].

Due to amylin's well-established effects on satiation, a focus on the modulation of the mesolimbic DA has centered around amylin's effects on feeding behavior. However, the findings that reward induced by food and addictive drugs involve common mechanisms, raise the possibility that amylin could be involved in reward regulation [124]. In this regard, sCT has been shown to attenuate the rewarding properties of alcohol in rodents, and more specifically to decrease alcohol-induced locomotor activity, NAc dopamine release, and conditioned place preference in mice, as well as alcohol intake in rats [125]. A better understanding of the effects of amylin activation on motivated behaviors could inform future approaches targeting amylin signaling to treat both behaviors and/or drug dependence.

5. Hypothalamic pathways

Amylin has long been promoted for anti-obesity treatment since trial experiments with its synthetic analogue pramlintide in outbred, diet-induced obese (DIO) rats resulted in sustained, fat-specific weight loss [126]. In addition to the reduced food intake of treated animals, pramlintide seemed to facilitate fat utilization and thus have beneficial effects beyond caloric restriction in obese animals [127]. These effects have been largely attributed to an improvement of leptin responsiveness, specifically in the ventromedial hypothalamus (VMH) [126]. Indeed, selectively bred DIO rats show a reduction in ^{125}I-amylin binding in the VMH that leads to a reduction of leptin-induced pSTAT3. This observation could be recapitulated by knocking down the amylin receptor in the VMH of DR rats, which resulted in reduced leptin signaling, increased adiposity, hyperleptinemia and hyperinsulinemia independent of food intake [14]. Amylin-deficient mice show a reduction of leptin receptor mRNA expression in the VMH and ARC, which results in reduced pSTAT3 and an attenuation of leptin-induced weight reduction [59]. The exact mechanism of amylin/leptin-interaction is still poorly understood but interestingly, hypothalamic microglia seem to produce IL-6 in response to amylin and possibly play an important role in increasing the ARC/VMH leptin response in both mice and rats through indirect modulation with cytokines [57]. Gene expression data from this study also showed that 5 days of systemic amylin treatment upregulates neuropeptide Y (NPY) and agouti-related protein (AgRP) in the ARC [57], while earlier studies with pramlintide showed increased POMC expression in rats [127]. This led us to the question: what effects occur from direct and leptin-independent amylin signaling onto these specialized neuronal populations in the ARC?

5.1. POMC neurons

Pro-opiomelanocortin (POMC) neurons are first order neurons in the hypothalamic control of body weight and reside mainly in the ARC [128]. Early observations of rat brains after chronic pramlintide treatment revealed an increase in POMC expression, but the predominant effects of amylin analogues on food intake are mediated by the AP and are not altered in mice that have inhibited melanocortin receptor signaling [127,129]. In the AP, activation of the amylin receptor on neurons leads to phosphorylation of extracellular-signal regulated kinase 1/2 (pERK), and blocking this intracellular pathway attenuates the inhibitory effect of amylin on food intake [44]. The same pathway seems to be activated when amylin acts on hypothalamic POMC neurons [22], where amylin was first hypothesized to converge with leptin signaling. CTR knockout studies in POMC neurons have shown that under physiological conditions, this pathway mainly mediates a thermogenic response, possibly promoting energy expenditure through activation of brown adipose tissue [18]. However, the effect of endogenous amylin on POMC neurons was no longer observable in 45 % HFD-fed knockout mice [18]. Additionally, mice that lack endogenous amylin also don't gain excess weight on HFD compared to wildtype [59]. Thus, the lack of endogenous amylin signaling in the ARC does not seem to worsen obesity under HFD conditions. Nevertheless, obese animals do not develop amylin resistance, since various animal models of obesity still respond well to pharmacological doses of amylin [59,130,131].

5.2. NPY neurons

In the classic model of hypothalamic body weight control, neuropeptide Y (NPY) neurons play the orexigenic counterpart to POMC neurons in the ARC [132,133]. While amylin can counteract pharmacological application of NPY and attenuate the orexigenic effect of NPY [134], the endogenous expression of NPY is sometimes unchanged or upregulated after amylin or pramlintide treatment [57,127,135]. When pERK expression in the hypothalamus after amylin injection was assessed, the increase in ERK phosphorylation was predominantly detected in POMC neurons and not NPY neurons in mice and rats [22]. However, tracking of fluorescently labeled sCT, an agonist for AMY and CTR, showed that NPY neurons and not POMC neurons are able to internalize this compound [31]. While NPY neurons express the necessary components of the amylin receptor, it is still unclear what the direct effect amylin on NPY neurons is, but one could hypothesize that it is of inhibitory nature and therefore overlooked in studies assessing neuronal activation. However, multiple factors are necessary to reverse the fasting-induced activation of ARC neurons, meaning that these first-order neurons already integrate several peripheral signals and endogenous amylin alone may not be sufficient to block NPY-driven feeding behavior [134].

5.3. Projections to second order neurons

Studies with unconditional amylin knockout mice and RAMP1/3-knockout mice revealed that a lack of amylin or amylin signaling during development results in a decrease of α-MSH fibers that project from the ARC to the PVN. Parallel projections of NPY neurons with AgRP-containing fibers are also reduced in RAMP1/3 KO mice, but increased in the amylin KO [22] which may suggest a different role between other AMY1/3 ligands and amylin. Generally, the presence of amylin seems to promote the outgrowth of pathways to second order neurons which it also activates under physiological conditions, e.g. the release of α-MSH

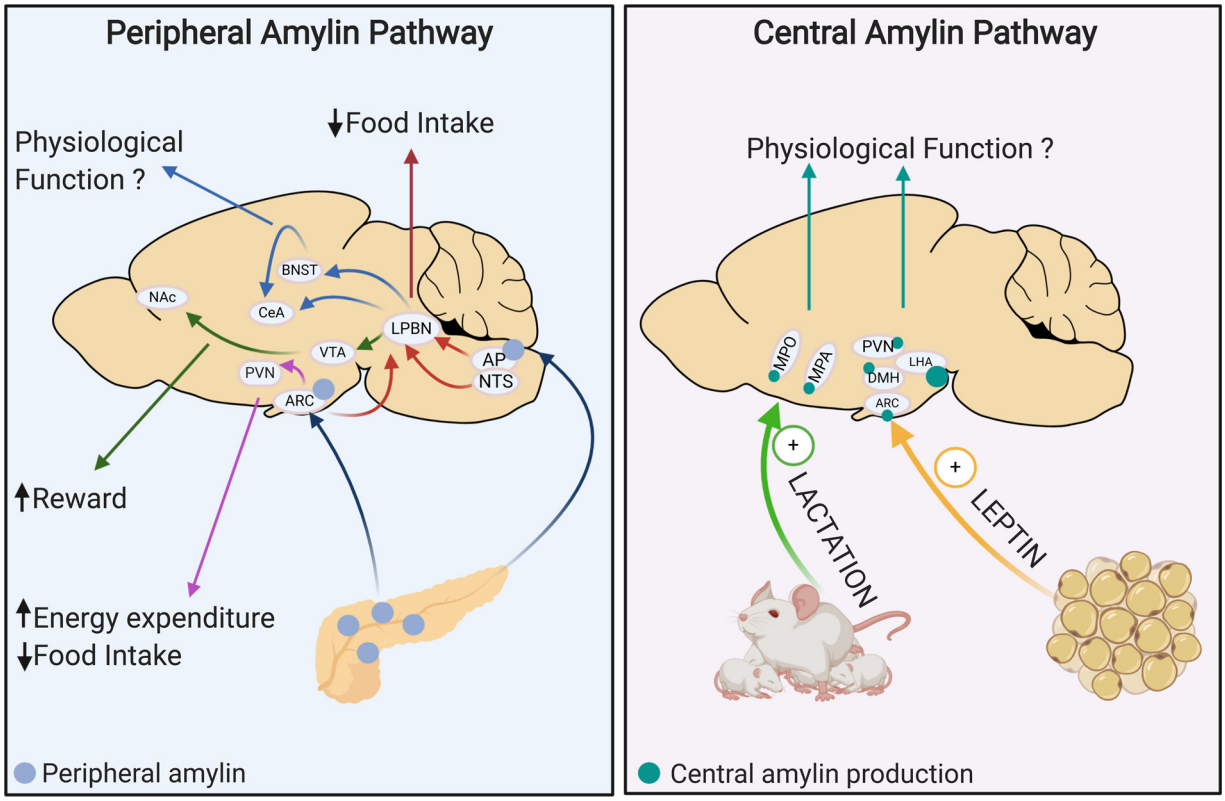

Fig. 1. Schematic overview of signaling pathways controlled by peripheral and central amylin. (**Left**) Peripheral amylin is secreted by pancreatic β-cells in response to nutrient intake. Amylin activates area postrema (AP)/nucleus of the solitary tract (NTS) neurons. Activation signal is then transmitted to downstream nuclei: activation of neurons of the lateral parabrachial nucleus (LPBN) decreases food intake while the activation of neurons of the ventral tegmental area (VTA) and the dopamine system mediates amylin's rewarding properties. Peripheral amylin can also bind to arcuate (ARC) neurons to decrease food intake and to increase energy expenditure. (**Right**) Amylin can be centrally produced by neurons of the medial preoptic nucleus (MPO), medial preoptic area (MPA) and multiple nuclei of the hypothalamus. Central amylin production is enhanced in lactating dams and is stimulated by leptin. Nevertheless, the physiological function of central amylin remains to be investigated.

to melanocortin-4 receptors (MC4R) [22,136]. Conversely, tamoxifen-induced knockout of CTR in POMC neurons of weaned mice did not affect the fiber density of α-MSH in the PVN [18]. These developmental effects could be important since they might change how the brain reacts to feeding-related signals later in life.

5.4. Do ARC and AP pathways join in LPBN?

Neurons located in the AP and ARC play both an important role in mediating amylin's anorectic effect and both nuclei project directly to the LPBN. Surgical AP removal is sufficient to decrease peripheral amylin's and sCT's hypophagic effect [96]. Conversely, systemic amylin administration activates ERK signaling in POMC neurons of the ARC through an AP independent pathway [22] and can suppress Agouti Related Peptide $AgRP^{ARC}$ neurons activity [137]. Indeed, $AgRP^{ARC}$ stimulation decreased the hyphophagic effect of exogenously applied amylin and reduced c-Fos expression in $CGRP^{LPBN}$ neurons [138]. Therefore, given the fact that the amylin receptor agonist, sCT, binds to ARC^{NPY}neurons [31], it is possible that amylin can also activate LPBN neurons by directly modulating ARC neurons via an AP-independent neuronal circuit.

Food intake is regulated by AgRP and POMC ARC neurons with AgRP neurons acting in opposition to POMC neurons. Indeed, the chemogenetic inhibition of AgRP neurons significantly decreases feeding [139] and genetic ablation of AgRP neurons causes severe starvation [140]. In contrast, stimulation of AgRP neurons determines an increase in food intake [141,142]. These neurons exert functions by primarily via GABAergic signaling onto POMC neurons [38,132,143,144] and also inhibit brain circuits that actively suppress appetite, thus increasing feeding. Indeed, $CGRP^{LPBN}$ neurons increased their activity following AgRP neuron ablation [38,143]. Further studies are needed to investigate the specific role of ARC signaling in the modulation of LPBN neural activity following amylin administration.

6. Mediation of gastric emptying and glucose control by systemic amylin

While the previous chapters concentrated on the effect of amylin on different brain nuclei, this last section will summarize findings on the peripheral effect of amylin through vagal efferent fibers as well as its role in regulating glucose secretion.

6.1. Amylin action on gastric emptying

Physiological concentrations of amylin have been shown to inhibit liver gluconeogenesis, food intake, and gastric emptying in people [145]. Many studies from our own laboratory and others have demonstrated that amylin activates AP neurons to perform its anorectic action; this activation seems to be direct and vagal or non-vagal afferents are not involved in mediating these effects [71,74–77,146].

Indeed, in rats, AMY and CTR agonists have been shown to decrease gastric emptying which contributes to their effect to decrease eating [73,147–149]. To assess the mechanisms of amylin on gastric emptying, a subdiaphragmatic vagal deafferentation procedure (SDA) was performed in rats. This procedure includes the unilateral severing of afferent fibers of the vagus where they enter the brain stem while a subdiaphragmatic vagotomy is performed on the contralateral side. Thus, even though amylin slowed gastric emptying in sham-operated

rats similarly to CCK, SDA did not attenuate gastric emptying in amylin-treated rats contrary to CKK-injected rats [81]. Furthermore, total subdiaphramagtic vagotomy [150] or lesion of the AP were able to block amylin-mediated gastric emptying [151]. These results indicate that amylin does not inhibit gastric emptying through vagal afferent [81] but rather through the AP and vagal efferent fibers. Furthermore, whole brain imaging revealed DMX fluorescent sCT binding [31] which may imply that amylin or amylin receptor agonist might also mediate gastric emptying by activating neurons of the DMX in addition to the AP.

Amylin receptors are also expressed in the stomach of humans and rats [152,153] but whether amylin is able to act on stomach muscles to slow gastric emptying has been scarcely assessed. A study by Mulder and colleagues [154] showed that amylin is able to relax gut muscle ex vivo which may imply that amylin could inhibit gastric emptying through this mechanism but whether this also takes place in vivo remains to be determined.

6.2. Amylin's role in the glucose control

The prevalence of type 2 diabetes increases concomitantly with the obesity epidemic and even though insulin therapies have improved, many patients still experience difficulties in the management of their glycemic control. Because under most circumstances, circulating amylin and insulin levels change in parallel, the amylin analogue pramlintide has been introduced as an adjunct therapy with insulin for diabetes therapy to improve glucose control in type 1 and type 2 diabetes patients [155]. Indeed, pramlintide injection in addition to mealtime insulin, delays gastric emptying and small intestin nutrient absorption, slowing the appearance of meal-derived glucose into the circulation and thus reducing postprandial glucose excursion in insulin requiring diabetic patients [156,157]. Nevertheless, pramlintide cannot reduce hyperglycemia following liquid glucose solution intake suggesting that its effects on glycemia are mostly linked to the slowing of gastric emptying [145,156,158].

Amylin has also been shown to act on pancreatic α-cells to inhibit glucagon secretion [158]. Indeed, hepatic glucose production and glycogenolysis are controlled by glucagon [159] and because type 1 and 2 diabetics fail to suppress postprandial glucagon secretion leading to inappropriate hepatic glucose production and hyperglycemia [160,161], amylin or its analog pramlintide reduce this maladaptation. Similarly to pramlintide, dual AMY and CTR agonist (DACRA) have been shown to exert positive effect on glucose metabolism in obese rodents [162,163]. Amylin's effects on glucagon release was assessed using hyperinsulinemic euglycemic clamp studies, and rats were exposed to arginine, a well-known non-glucose secretagogue, followed by hypoglycemia to stimulate glucagon secretion. Amylin was able to inhibit arginine-induced but not hypoglycemia-induced glucagon release [80,164]. Since these effects require an intact animal and are not present in isolated rodents islets [165], it implies that amylin action on glucagon secretion is extrapancreatic and may be mediated by AP signaling and the autonomic nervous system.

7. Conclusion

To conclude, amylin signals into multiple fore- and hindbrain areas to perform a variety of physiological effects (Fig. 1). Amylin's main metabolic effects are triggered by the activation of AP neurons which then propagate the signal to downstream nuclei. While AP neuronal activation is well characterized, the actions mediated by its downstream nuclei, and whether forebrain and hindbrain signaling pathways meet at the level of the LBPN remains to be clarified. Furthermore, this review focused amylin brain circuitry in rodents, whether this is also true in human needs to be assessed given the fact that human amylin can form aggregates.

Nevertheless, given the number of physiological functions under amylin control, the development of an amylin agonist could be a viable pharmacological option to prevent obesity.

References

[1] T.A. Lutz, J.S. Rand, Detection of amyloid deposition in various regions of the feline pancreas by different staining techniques, J. Comp. Pathol. 116 (2) (1997) 157–170.
[2] G.J. Cooper, Amylin compared with calcitonin gene-related peptide: structure, biology, and relevance to metabolic disease, Endocr. Rev. 15 (2) (1994) 163–201.
[3] T.A. Lutz, Effects of amylin on eating and adiposity, Handbook of Experimental Pharmacology (209), (2012), pp. 231–250.
[4] C.N. Boyle, T.A. Lutz, C. Le Foll, Amylin - its role in the homeostatic and hedonic control of eating and recent developments of amylin analogs to treat obesity, Mol. Metab. 8 (2018) 203–210.
[5] B.E. Levin, T.A. Lutz, Amylin and leptin: co-regulators of energy homeostasis and neuronal development, Trends Endocrinol. Metab. 28 (2) (2017) 153–164.
[6] R.L. Bower, D.L. Hay, Amylin structure-function relationships and receptor pharmacology: implications for amylin mimetic drug development, Br. J. Pharmacol. 173 (12) (2016) 1883–1898.
[7] P. Westermark, A. Andersson, G.T. Westermark, Islet amyloid polypeptide, islet amyloid, and diabetes mellitus, Physiol. Rev. 91 (3) (2011) 795–826.
[8] Z. Li, L. Kelly, M. Heiman, P. Greengard, J.M. Friedman, Hypothalamic amylin acts in concert with leptin to regulate food intake, Cell Metab. 23 (5) (2016) 945.
[9] A. Dobolyi, Central amylin expression and its induction in rat dams, J. Neurochem. 111 (6) (2009) 1490–1500.
[10] D.L. Hay, M.L. Garelja, D.R. Poyner, C.S. Walker, Update on the pharmacology of calcitonin/CGRP family of peptides: IUPHAR review 25, Br. J. Pharmacol. 175 (1) (2018) 3–17.
[11] H. Nakamoto, Y. Soeda, S. Takami, M. Minami, M. Satoh, Localization of calcitonin receptor mRNA in the mouse brain: coexistence with serotonin transporter mRNA, brain research, Mol. Brain. Res. 76 (1) (2000) 93–102.
[12] C. Becskei, T. Riediger, D. Zund, P. Wookey, T.A. Lutz, Immunohistochemical mapping of calcitonin receptors in the adult rat brain, Brain Res. 1030 (2) (2004) 221–233.
[13] P.M. Sexton, J.S. McKenzie, R.T. Mason, J.M. Moseley, T.J. Martin, F.A. Mendelsohn, Localization of binding sites for calcitonin gene-related peptide in rat brain by in vitro autoradiography, Neuroscience 19 (4) (1986) 1235–1245.
[14] A.A. Dunn-Meynell, C. Le Foll, M.D. Johnson, T.A. Lutz, M.R. Hayes, B.E. Levin, Endogenous VMH amylin signaling is required for full leptin signaling and protection from diet-induced obesity, Am. J. Physiol. Regul. Integr. Compar. Physiol. 310 (4) (2016) R355–65.
[15] D.R. Poyner, P.M. Sexton, I. Marshall, D.M. Smith, R. Quirion, W. Born, R. Muff, J.A. Fischer, S.M. Foord, International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors, Pharmacol. Rev. 54 (2) (2002) 233–246.
[16] E.E. Moore, R.E. Kuestner, S.D. Stroop, F.J. Grant, S.L. Matthewes, C.L. Brady, P.M. Sexton, D.M. Findlay, Functionally different isoforms of the human calcitonin receptor result from alternative splicing of the gene transcript, Mol. Endocrinol. 9 (8) (1995) 959–968.
[17] E.G. Mietlicki-Baase, L.E. Rupprecht, D.R. Olivos, D.J. Zimmer, M.D. Alter, R.C. Pierce, H.D. Schmidt, M.R. Hayes, Amylin receptor signaling in the ventral tegmental area is physiologically relevant for the control of food intake, Neuropsychopharmacology 38 (9) (2013) 1685–1697.
[18] B. Coester, C. Koester-Hegmann, T.A. Lutz, C. Le Foll, Amylin/calcitonin receptor-mediated signaling in POMC neurons influences energy balance and locomotor activity in chow-fed male mice, Diabetes (2020).
[19] K. Husmann, P.M. Sexton, J.A. Fischer, W. Born, Mouse receptor-activity-modifying proteins 1, -2 and -3: amino acid sequence, expression and function, Mol. Cell. Endocrinol. 162 (1–2) (2000) 35–43.
[20] T. Ueda, S. Ugawa, Y. Saishin, S. Shimada, Expression of receptor-activity modifying protein (RAMP) mRNAs in the mouse brain, brain research, Mol. Brain. Res. 93 (1) (2001) 36–45.
[21] T.J. Stachniak, T.L. Krukoff, Receptor activity modifying protein 2 distribution in the rat central nervous system and regulation by changes in blood pressure, J. Neuroendocrinol. 15 (9) (2003) 840–850.
[22] T.A. Lutz, B. Coester, L. Whiting, A.A. Dunn-Meynell, C.N. Boyle, S.G. Bouret, B.E. Levin, C. Le Foll, Amylin selectively signals onto POMC neurons in the arcuate nucleus of the hypothalamus, Diabetes 67 (5) (2018) 805–817.
[23] M. Kadmiel, K.L. Fritz-Six, K.M. Caron, Understanding RAMPs through genetically engineered mouse models, Adv. Exp. Med. Biol. 744 (2012) 49–60.
[24] B. Coester, S.W. Pence, S. Arrigoni, C.N. Boyle, C. Le Foll, T.A. Lutz, RAMP1 and RAMP3 differentially control amylin's effects on food intake, glucose and energy balance in male and female mice, Neuroscience (2019).
[25] C. Thota, P.R. Gangula, Y.L. Dong, C. Yallampalli, Changes in the expression of calcitonin receptor-like receptor, receptor activity-modifying protein (RAMP) 1, RAMP2, and RAMP3 in rat uterus during pregnancy, labor, and by steroid hormone treatments, Biol. Reprod. 69 (4) (2003) 1432–1437.
[26] M. Kadmiel, K. Fritz-Six, S. Pacharne, G.O. Richards, M. Li, T.M. Skerry, K.M. Caron, Research resource: haploinsufficiency of receptor activity-modifying protein-2 (RAMP2) causes reduced fertility, hyperprolactinemia, skeletal abnormalities, and endocrine dysfunction in mice, Mol. Endocrinol. 25 (7) (2011) 1244–1253.
[27] M.L. Garelja, C.A. Walker, A. Siow, S.H. Yang, P.W.R. Harris, M.A. Brimble,

H.A. Watkins, J.J. Gingell, D.L. Hay, receptor activity modifying proteins have limited effects on the class B G protein-coupled receptor calcitonin receptor-like receptor stalk, Biochemistry 57 (8) (2018) 1410–1422.
[28] C.G. Liberini, C.N. Boyle, C. Cifani, M. Venniro, B.T. Hope, T.A. Lutz, Amylin receptor components and the leptin receptor are co-expressed in single rat area postrema neurons, Eur. J. Neurosci. 43 (5) (2016) 653–661.
[29] P.M. Sexton, G. Paxinos, M.A. Kenney, P.J. Wookey, K. Beaumont, In vitro autoradiographic localization of amylin binding sites in rat brain, Neuroscience 62 (2) (1994) 553–567.
[30] K. Beaumont, M.A. Kenney, A.A. Young, T.J. Rink, High affinity amylin binding sites in rat brain, Mol. Pharmacol. 44 (3) (1993) 493–497.
[31] H.L. Zakariassen, L.M. John, J. Lykkesfeldt, K. Raun, T. Glendorf, L. Schaffer, S. Lundh, A. Secher, T.A. Lutz, C. Le Foll, Salmon calcitonin distributes into the arcuate nucleus to a subset of NPY neurons in mice, Neuropharmacology 167 (2020) 107987.
[32] G. Christopoulos, G. Paxinos, X.F. Huang, K. Beaumont, A.W. Toga, P.M. Sexton, Comparative distribution of receptors for amylin and the related peptides calcitonin gene related peptide and calcitonin in rat and monkey brain, Can. J. Physiol. Pharmacol. 73 (7) (1995) 1037–1041.
[33] G. Christopoulos, K.J. Perry, M. Morfis, N. Tilakaratne, Y. Gao, N.J. Fraser, M.J. Main, S.M. Foord, P.M. Sexton, Multiple amylin receptors arise from receptor activity-modifying protein interaction with the calcitonin receptor gene product, Mol. Pharmacol. 56 (1) (1999) 235–242.
[34] D.L. Hay, S. Chen, T.A. Lutz, D.G. Parkes, J.D. Roth, Amylin: pharmacology, physiology, and clinical potential, Pharmacol. Rev. 67 (3) (2015) 564–600.
[35] J.J. Gingell, J. Simms, J. Barwell, D.R. Poyner, H.A. Watkins, A.A. Pioszak, P.M. Sexton, D.L. Hay, Erratum: an allosteric role for receptor activity-modifying proteins in defining GPCR pharmacology, Cell Discov. 2 (2016) 16020.
[36] C.S. Walker, S. Eftekhari, R.L. Bower, A. Wilderman, P.A. Insel, L. Edvinsson, H.J. Waldvogel, M.A. Jamaluddin, A.F. Russo, D.L. Hay, A second trigeminal CGRP receptor: function and expression of the AMY1 receptor, Ann. Clin. Transl. Neurol. 2 (6) (2015) 595–608.
[37] M.E. Carter, M.E. Soden, L.S. Zweifel, R.D. Palmiter, Genetic identification of a neural circuit that suppresses appetite, Nature 503 (7474) (2013) 111–114.
[38] C.A. Campos, A.J. Bowen, M.W. Schwartz, R.D. Palmiter, Parabrachial CGRP neurons control meal termination, Cell Metab. 23 (5) (2016) 811–820.
[39] M.E. Carter, S. Han, R.D. Palmiter, Parabrachial calcitonin gene-related peptide neurons mediate conditioned taste aversion, J. Neurosci. 35 (11) (2015) 4582–4586.
[40] T.A. Lutz, R. Rossi, J. Althaus, E. Del Prete, E. Scharrer, Amylin reduces food intake more potently than calcitonin gene-related peptide (CGRP) when injected into the lateral brain ventricle in rats, Peptides 19 (9) (1998) 1533–1540.
[41] D.L. Hay, D.R. Poyner, P.M. Sexton, GPCR modulation by RAMPs, Pharmacol. Ther. 109 (1–2) (2006) 173–197.
[42] T. Riediger, H.A. Schmid, T. Lutz, E. Simon, Amylin potently activates AP neurons possibly via formation of the excitatory second messenger cGMP, Am. J. Physiol. Regul. Integr. Comp. Physiol. 281 (6) (2001) R1833–43.
[43] A. Mollet, S. Gilg, T. Riediger, T.A. Lutz, Infusion of the amylin antagonist AC 187 into the area postrema increases food intake in rats, Physiol. Behav. 81 (1) (2004) 149–155.
[44] C.S. Potes, C.N. Boyle, P.J. Wookey, T. Riediger, T.A. Lutz, Involvement of the extracellular signal-regulated kinase 1/2 signaling pathway in amylin's eating inhibitory effect, Am. J. Physiol. Regul. Integr. Comp. Physiol. 302 (3) (2012) R340–51.
[45] W.A. Banks, A.J. Kastin, Differential permeability of the blood-brain barrier to two pancreatic peptides: insulin and amylin, Peptides 19 (5) (1998) 883–889.
[46] W.A. Banks, A.J. Kastin, L.M. Maness, W. Huang, J.B. Jaspan, Permeability of the blood-brain barrier to amylin, Life Sci. 57 (22) (1995) 1993–2001.
[47] N. Schultz, S. Janelidze, E. Byman, L. Minthon, K. Nagga, O. Hansson, M. Wennstrom, Levels of islet amyloid polypeptide in cerebrospinal fluid and plasma from patients with Alzheimer's disease, PLoS one 14 (6) (2019) e0218561.
[48] E. Balland, J. Dam, F. Langlet, E. Caron, S. Steculorum, A. Messina, S. Rasika, A. Falluel-Morel, Y. Anouar, B. Dehouck, E. Trinquet, R. Jockers, S.G. Bouret, V. Prevot, Hypothalamic tanycytes are an ERK-gated conduit for leptin into the brain, Cell Metab. 19 (2) (2014) 293–301.
[49] B.P. Roques, Neuropeptide inactivation or metabolism, in: L.R. Squire (Ed.), Encyclopedia of Neuroscience, Academic Press, Oxford, 2009, pp. 789–799.
[50] J.A. Fischer, P.H. Tobler, H. Henke, F.A. Tschopp, Salmon and human calcitonin-like peptides coexist in the human thyroid and brain, J. Clin. Endocrinol. Metab. 57 (6) (1983) 1314–1316.
[51] G. Skofitsch, S.J. Wimalawansa, D.M. Jacobowitz, W. Gubisch, Comparative immunohistochemical distribution of amylin-like and calcitonin gene related peptide like immunoreactivity in the rat central nervous system, Can. J. Physiol. Pharmacol. 73 (7) (1995) 945–956.
[52] P.M. Sexton, J.M. Hilton, Biologically active salmon calcitonin-like peptide is present in rat brain, Brain Res. 596 (1–2) (1992) 279–284.
[53] M.L. Forsling, Regulation of oxytocin release, in: B. Springer (Ed.), Heidelberg, 1986, pp. 19–53.
[54] P. Gray, P.J. Brooks, Effect of lesion location within the medial preoptic-anterior hypothalamic continuum on maternal and male sexual behaviors in female rats, Behav. Neurosci. 98 (4) (1984) 703–711.
[55] M. Numan, Neural basis of maternal behavior in the rat, Psychoneuroendocrinology 13 (1-2) (1988) 47–62.
[56] A.C. Ribeiro, G. Ceccarini, C. Dupre, J.M. Friedman, D.W. Pfaff, A.L. Mark, Contrasting effects of leptin on food anticipatory and total locomotor activity, PLoS one 6 (8) (2011) e23364.
[57] C. Le Foll, M.D. Johnson, A.A. Dunn-Meynell, C.N. Boyle, T.A. Lutz, B.E. Levin, Amylin-induced central IL-6 production enhances ventromedial hypothalamic leptin signaling, Diabetes 64 (5) (2015) 1621–1631.
[58] J.L. Trevaskis, T. Coffey, R. Cole, C. Lei, C. Wittmer, B. Walsh, C. Weyer, J. Koda, A.D. Baron, D.G. Parkes, J.D. Roth, Amylin-mediated restoration of leptin responsiveness in diet-induced obesity: magnitude and mechanisms, Endocrinology 149 (11) (2008) 5679–5687.
[59] V.F. Turek, J.L. Trevaskis, B.E. Levin, A.A. Dunn-Meynell, B. Irani, G. Gu, C. Wittmer, P.S. Griffin, C. Vu, D.G. Parkes, J.D. Roth, Mechanisms of amylin/leptin synergy in rodent models, Endocrinology 151 (1) (2010) 143–152.
[60] J.D. Roth, H. Maier, S. Chen, B.L. Roland, Implications of amylin receptor agonism: integrated neurohormonal mechanisms and therapeutic applications, Arch. Neurol. 66 (3) (2009) 306–310.
[61] H.S. Moon, J.P. Chamberland, K.N. Diakopoulos, C.G. Fiorenza, F. Ziemke, B. Schneider, C.S. Mantzoros, Leptin and amylin act in an additive manner to activate overlapping signaling pathways in peripheral tissues: in vitro and ex vivo studies in humans, Diabetes care 34 (1) (2011) 132–138.
[62] M. Mirza, M.D. J, Neuroanatomy, Area Postrema, StatPearls, Treasure Island (FL), 2020.
[63] G.B. Wislocki, E.H. Leduc, Vital staining of the hematoencephalic barrier by silver nitrate and trypan blue, and cytological comparisons of the neurohypophysis, pineal body, area postrema, intercolumnar tubercle and supraoptic crest, J. Comp. Neurol. 96 (3) (1952) 371–413.
[64] D.K. Morest, Experimental study of the projections of the nucleus of the tractus solitarius and the area postrema in the cat, J. Comp. Neurol. 130 (4) (1967) 277–300.
[65] K.E. Astrom, A.P. Scand (Ed.), On the Central Course of Afferent Fibres in the Trigeminal, Facial, Glossopharyngeal, and Vagal Nerves and Their Nuclei in the Mouse, 1953, pp. 209–320.
[66] R.J. Contreras, R.M. Beckstead, R. Norgren, The central projections of the trigeminal, facial, glossopharyngeal and vagus nerves: an autoradiographic study in the rat, J. Auton. Nerv. Syst. 6 (3) (1982) 303–322.
[67] A. Torvik, Afferent connections to the sensory trigeminal nuclei, the nucleus of the solitary tract and adjacent structures; An experimental study in the rat, J. Comp. Neurol. 106 (1) (1956) 51–141.
[68] M. Kalia, M.M. Mesulam, Brain stem projections of sensory and motor components of the vagus complex in the cat: II. Laryngeal, tracheobronchial, pulmonary, cardiac, and gastrointestinal branches, J. Comp. Neurol. 193 (2) (1980) 467–508.
[69] M. Kalia, R.V. Welles, Brain stem projections of the aortic nerve in the cat: a study using tetramethyl benzidine as the substrate for horseradish peroxidase, Brain Res. 188 (1) (1980) 23–32.
[70] R.J. Contreras, E. Fox, M.L. Drugovich, Area postrema lesions produce feeding deficits in the rat: effects of preoperative dieting and 2-deoxy-D-glucose, Physiol. Behav. 29 (5) (1982) 875–884.
[71] T.A. Lutz, M. Senn, J. Althaus, E. Del Prete, F. Ehrensperger, E. Scharrer, Lesion of the area postrema/nucleus of the solitary tract (AP/NTS) attenuates the anorectic effects of amylin and calcitonin gene-related peptide (CGRP) in rats, Peptides 19 (2) (1998) 309–317.
[72] C. Fernandes-Santos, Z. Zhang, D.A. Morgan, D.F. Guo, A.F. Russo, K. Rahmouni, Amylin acts in the central nervous system to increase sympathetic nerve activity, Endocrinology 154 (7) (2013) 2481–2488.
[73] C.M. Mack, C.J. Soares, J.K. Wilson, J.R. Athanacio, V.F. Turek, J.L. Trevaskis, J.D. Roth, P.A. Smith, B. Gedulin, C.M. Jodka, B.L. Roland, S.H. Adams, A. Lwin, J. Herich, K.D. Laugero, C. Vu, R. Pittner, J.R. Paterniti, M. Hanley, S. Ghosh, D.G. Parkes, Davalintide (AC2307), a novel amylin-mimetic peptide: enhanced pharmacological properties over native amylin to reduce food intake and body weight, Int. J. Obes. 34 (2) (2010) 385–395.
[74] J.E. Morley, J.F. Flood, M. Horowitz, P.M. Morley, M.J. Walter, Modulation of food intake by peripherally administered amylin, Am. J. Physiol. 267 (1 Pt 2) (1994) R178–84.
[75] T.A. Lutz, A. Mollet, P.A. Rushing, T. Riediger, E. Scharrer, The anorectic effect of a chronic peripheral infusion of amylin is abolished in area postrema/nucleus of the solitary tract (AP/NTS) lesioned rats, Int. J. Obes. Relat. Metab. Disord.: J. Int. Assoc. Study Obes. 25 (7) (2001) 1005–1011.
[76] T.A. Lutz, E. Del Prete, E. Scharrer, Subdiaphragmatic vagotomy does not influence the anorectic effect of amylin, Peptides 16 (3) (1995) 457–462.
[77] T.A. Lutz, E. Del Prete, E. Scharrer, Reduction of food intake in rats by intraperitoneal injection of low doses of amylin, Physiol. Behav. 55 (5) (1994) 891–895.
[78] T. Riediger, D. Zuend, C. Becskei, T.A. Lutz, The anorectic hormone amylin contributes to feeding-related changes of neuronal activity in key structures of the gut-brain axis, Am. J. Physiol. Regul. Integr. Comp. Physiol. 286 (1) (2004) R114–22.
[79] T. Riediger, H.A. Schmid, T.A. Lutz, E. Simon, Amylin and glucose co-activate area postrema neurons of the rat, Neurosci. Lett. 328 (2) (2002) 121–124.
[80] B.R. Gedulin, T.J. Rink, A.A. Young, Dose-response for glucagonostatic effect of amylin in rats, Metabolism 46 (1) (1997) 67–70.
[81] J. Wickbom, M.K. Herrington, J. Permert, A. Jansson, U. Arnelo, Gastric emptying in response to IAPP and CCK in rats with subdiaphragmatic afferent vagotomy, Regul. Pept. 148 (1–3) (2008) 21–25.
[82] A.A. Young, B. Gedulin, W. Vine, A. Percy, T.J. Rink, Gastric emptying is accelerated in diabetic BB rats and is slowed by subcutaneous injections of amylin, Diabetologia 38 (6) (1995) 642–648.
[83] F.E. Braegger, L. Asarian, K. Dahl, T.A. Lutz, C.N. Boyle, The role of the area postrema in the anorectic effects of amylin and salmon calcitonin: behavioral and neuronal phenotyping, Eur. J. Neurosci. 40 (7) (2014) 3055–3066.
[84] C.S. Potes, V.F. Turek, R.L. Cole, C. Vu, B.L. Roland, J.D. Roth, T. Riediger,

T.A. Lutz, Noradrenergic neurons of the area postrema mediate amylin's hypophagic action, Am. J. Physiol. Regul. Integr. Comp. Physiol. 299 (2) (2010) R623–31.
[85] T. Fukuda, Y. Hirai, H. Maezawa, Y. Kitagawa, M. Funahashi, Electrophysiologically identified presynaptic mechanisms underlying amylinergic modulation of area postrema neuronal excitability in rat brain slices, Brain Res. 1494 (2013) 9–16.
[86] H.L. Borison, R. Borison, L.E. McCarthy, Role of the area postrema in vomiting and related functions, Fed. Proc. 43 (15) (1984) 2955–2958.
[87] K. Shinpo, Y. Hirai, H. Maezawa, Y. Totsuka, M. Funahashi, The role of area postrema neurons expressing H-channels in the induction mechanism of nausea and vomiting, Physiol. Behav. 107 (1) (2012) 98–103.
[88] G. Tsukamoto, A. Adachi, Neural responses of rat area postrema to stimuli producing nausea, J. Auton. Nerv. Syst. 49 (1) (1994) 55–60.
[89] A.D. Miller, R.A. Leslie, The area postrema and vomiting, Front. Neuroendocrinol. 15 (4) (1994) 301–320.
[90] P.A. Rushing, R.J. Seeley, E.L. Air, T.A. Lutz, S.C. Woods, Acute 3rd-ventricular amylin infusion potently reduces food intake but does not produce aversive consequences, Peptides 23 (5) (2002) 985–988.
[91] C. Mack, J. Wilson, J. Athanacio, J. Reynolds, K. Laugero, S. Guss, C. Vu, J. Roth, D. Parkes, Pharmacological actions of the peptide hormone amylin in the long-term regulation of food intake, food preference, and body weight, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (5) (2007) R1855–63.
[92] W.T. Chance, A. Balasubramaniam, X. Chen, J.E. Fischer, Tests of adipsia and conditioned taste aversion following the intrahypothalamic injection of amylin, Peptides 13 (5) (1992) 961–964.
[93] W. Cheng, I. Gonzalez, W. Pan, A.H. Tsang, J. Adams, E. Ndoka, D. Gordian, B. Khoury, K. Roelofs, S.S. Evers, A. MacKinnon, S. Wu, H. Frikke-Schmidt, J.N. Flak, J.L. Trevaskis, C.J. Rhodes, S.I. Fukada, R.J. Seeley, D.A. Sandoval, D.P. Olson, C. Blouet, M.G. Myers Jr., Calcitonin receptor neurons in the mouse nucleus tractus solitarius control energy balance via the Non-aversive suppression of feeding, Cell Metab. 31 (2) (2020) 301–312 e5.
[94] D. Zuger, K. Forster, T.A. Lutz, T. Riediger, Amylin and GLP-1 target different populations of area postrema neurons that are both modulated by nutrient stimuli, Physiol. Behav. 112-113 (2013) 61–69.
[95] C. Becskei, V. Grabler, G.L. Edwards, T. Riediger, T.A. Lutz, Lesion of the lateral parabrachial nucleus attenuates the anorectic effect of peripheral amylin and CCK, Brain Res. 1162 (2007) 76–84.
[96] T.R. Scott, D.M. Small, The role of the parabrachial nucleus in taste processing and feeding, Ann. N.Y. Acad. Sci. 1170 (2009) 372–377.
[97] H. Herbert, M.M. Moga, C.B. Saper, Connections of the parabrachial nucleus with the nucleus of the solitary tract and the medullary reticular formation in the rat, J. Comp. Neurol. 293 (4) (1990) 540–580.
[98] R.M. Beckstead, J.R. Morse, R. Norgren, The nucleus of the solitary tract in the monkey: projections to the thalamus and brain stem nuclei, J. Comp. Neurol. 190 (2) (1980) 259–282.
[99] J.A. Ricardo, E.T. Koh, Anatomical evidence of direct projections from the nucleus of the solitary tract to the hypothalamus, amygdala, and other forebrain structures in the rat, Brain Res. 153 (1) (1978) 1–26.
[100] Y. Kawai, K. Takami, S. Shiosaka, P.C. Emson, C.J. Hillyard, S. Girgis, I. MacIntyre, M. Tohyama, Topographic localization of calcitonin gene-related peptide in the rat brain: an immunohistochemical analysis, Neuroscience 15 (3) (1985) 747–763.
[101] R.D. Palmiter, The parabrachial nucleus: CGRP neurons function as a general alarm, Trends Neurosci. 41 (5) (2018) 280–293.
[102] D.M. Armstrong, V.M. Pickel, T.H. Joh, D.J. Reis, R.J. Miller, Immunocytochemical localization of catecholamine synthesizing enzymes and neuropeptides in area postrema and medial nucleus tractus solitarius of rat brain, J. Comp. Neurol. 196 (3) (1981) 505–517.
[103] T.A. Milner, T.H. Joh, V.M. Pickel, Tyrosine hydroxylase in the rat parabrachial region: ultrastructural localization and extrinsic sources of immunoreactivity, J. Neurosci. 6 (9) (1986) 2585–2603.
[104] M.S. Bienkowski, L. Rinaman, Common and distinct neural inputs to the medial central nucleus of the amygdala and anterior ventrolateral bed nucleus of stria terminalis in rats, Brain Struct. Funct. 218 (1) (2013) 187–208.
[105] B. Roozendaal, B.S. McEwen, S. Chattarji, Stress, memory and the amygdala, nature reviews, Neuroscience 10 (6) (2009) 423–433.
[106] E.A. Phelps, J.E. LeDoux, Contributions of the amygdala to emotion processing: from animal models to human behavior, Neuron 48 (2) (2005) 175–187.
[107] P. Veinante, I. Yalcin, M. Barrot, The amygdala between sensation and affect: a role in pain, J. Mol. Psychiatry 1 (1) (2013) 9.
[108] A. Sclafani, K. Touzani, R.J. Bodnar, Dopamine and learned food preferences, Physiol. Behav. 104 (1) (2011) 64–68.
[109] J.L. Price, A.N.Y.A. Sci (Ed.), Comparative Aspects of Amygdala Connectivity, 2006, pp. 50–58.
[110] M. Alvarez-Crespo, K.P. Skibicka, I. Farkas, C.S. Molnar, E. Egecioglu, E. Hrabovszky, Z. Liposits, S.L. Dickson, The amygdala as a neurobiological target for ghrelin in rats: neuroanatomical, electrophysiological and behavioral evidence, PLoS one 7 (10) (2012) e46321.
[111] I. Merchenthaler, M. Lane, P. Shughrue, Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system, J. Comp. Neurol. 403 (2) (1999) 261–280.
[112] R.H. Anderberg, C. Anefors, F. Bergquist, H. Nissbrandt, K.P. Skibicka, Dopamine signaling in the amygdala, increased by food ingestion and GLP-1, regulates feeding behavior, Physiol. Behav. 136 (2014) 135–144.
[113] H. Cai, W. Haubensak, T.E. Anthony, D.J. Anderson, Central amygdala PKC-delta (+) neurons mediate the influence of multiple anorexigenic signals, Nat. Neurosci. 17 (9) (2014) 1240–1248.
[114] J.E. Richard, R.H. Anderberg, A. Goteson, F.M. Gribble, F. Reimann, K.P. Skibicka, Activation of the GLP-1 receptors in the nucleus of the solitary tract reduces food reward behavior and targets the mesolimbic system, PLoS one 10 (3) (2015) e0119034.
[115] E.G. Mietlicki-Baase, L.E. McGrath, K. Koch-Laskowski, J. Krawczyk, D.J. Reiner, T. Pham, C.T.N. Nguyen, C.A. Turner, D.R. Olivos, M.E. Wimmer, H.D. Schmidt, M.R. Hayes, Amylin receptor activation in the ventral tegmental area reduces motivated ingestive behavior, Neuropharmacology 123 (2017) 67–79.
[116] H.R. Berthoud, Homeostatic and non-homeostatic pathways involved in the control of food intake and energy balance, Obesity 14 (Suppl 5) (2006) 197S–200S.
[117] C.M. Mack, P.A. Smith, J.R. Athanacio, K. Xu, J.K. Wilson, J.M. Reynolds, C.M. Jodka, M.G. Lu, D.G. Parkes, Glucoregulatory effects and prolonged duration of action of davalintide: a novel amylinomimetic peptide, Diabetes Obes. Metab. 13 (12) (2011) 1105–1113.
[118] A.N. Suarez, C.M. Liu, A.M. Cortella, E.E. Noble, S.E. Kanoski, Ghrelin and orexin interact to increase meal size through a descending hippocampus to hindbrain signaling pathway, Biol. Psychiatry (2019).
[119] S.E. Kanoski, M.R. Hayes, K.P. Skibicka, GLP-1 and weight loss: unraveling the diverse neural circuitry, Am. J. Physiol. Regul. Integr. Comp. Physiol. 310 (10) (2016) R885–95.
[120] L. Whiting, J.E. McCutcheon, C.N. Boyle, M.F. Roitman, T.A. Lutz, The area postrema (AP) and the parabrachial nucleus (PBN) are important sites for salmon calcitonin (sCT) to decrease evoked phasic dopamine release in the nucleus accumbens (NAc), Physiol. Behav. 176 (2017) 9–16.
[121] E.G. Mietlicki-Baase, D.J. Reiner, J.J. Cone, D.R. Olivos, L.E. McGrath, D.J. Zimmer, M.F. Roitman, M.R. Hayes, Amylin modulates the mesolimbic dopamine system to control energy balance, Neuropsychopharmacology 40 (2) (2015) 372–385.
[122] D.J. Reiner, E.G. Mietlicki-Baase, D.R. Olivos, L.E. McGrath, D.J. Zimmer, K. Koch-Laskowski, J. Krawczyk, C.A. Turner, E.E. Noble, J.D. Hahn, H.D. Schmidt, S.E. Kanoski, M.R. Hayes, Amylin acts in the lateral dorsal tegmental nucleus to regulate energy balance through gamma-aminobutyric acid signaling, Biol. Psychiatry 82 (11) (2017) 828–838.
[123] E.G. Mietlicki-Baase, M.R. Hayes, Amylin activates distributed CNS nuclei to control energy balance, Physiol. Behav. 136 (2014) 39–46.
[124] N.M. Avena, P. Rada, B.G. Hoebel, Underweight rats have enhanced dopamine release and blunted acetylcholine response in the nucleus accumbens while bingeing on sucrose, Neuroscience 156 (4) (2008) 865–871.
[125] A.L. Kalafateli, D. Vallof, E. Jerlhag, Activation of amylin receptors attenuates alcohol-mediated behaviours in rodents, Addict. Biol. 24 (3) (2019) 388–402.
[126] J.D. Roth, B.L. Roland, R.L. Cole, J.L. Trevaskis, C. Weyer, J.E. Koda, C.M. Anderson, D.G. Parkes, A.D. Baron, Leptin responsiveness restored by amylin agonism in diet-induced obesity: evidence from nonclinical and clinical studies, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 7257–7262.
[127] J.D. Roth, H. Hughes, E. Kendall, A.D. Baron, C.M. Anderson, Antiobesity effects of the beta-cell hormone amylin in diet-induced obese rats: effects on food intake, body weight, composition, energy expenditure, and gene expression, Endocrinology 147 (12) (2006) 5855–5864.
[128] M.W. Schwartz, S.C. Woods, D. Porte Jr., R.J. Seeley, D.G. Baskin, Central nervous system control of food intake, Nature 404 (6778) (2000) 661–671.
[129] F. Bolze, M. Klingenspor, Mouse models for the central melanocortin system, Genes Nutr 4 (2) (2009) 129–134.
[130] C.N. Boyle, T.A. Lutz, Amylinergic control of food intake in lean and obese rodents, Physiol. Behav. 105 (1) (2011) 129–137.
[131] C.N. Boyle, M.M. Rossier, T.A. Lutz, Influence of high-fat feeding, diet-induced obesity, and hyperamylinemia on the sensitivity to acute amylin, Physiol. Behav. 104 (1) (2011) 20–28.
[132] J.N. Betley, Z.F. Cao, K.D. Ritola, S.M. Sternson, Parallel, redundant circuit organization for homeostatic control of feeding behavior, Cell 155 (6) (2013) 1337–1350.
[133] Z. He, Y. Gao, A.L. Alhadeff, C.M. Castorena, Y. Huang, L. Lieu, S. Afrin, J. Sun, J.N. Betley, H. Guo, K.W. Williams, Cellular and synaptic reorganization of arcuate NPY/AgRP and POMC neurons after exercise, Mol. Metab. 18 (2018) 107–119.
[134] C. Becskei, T.A. Lutz, T. Riediger, Diet-derived nutrients mediate the inhibition of hypothalamic NPY neurons in the arcuate nucleus of mice during refeeding, Am. J. Physiol. Regul. Integr. Comp. Physiol. 297 (1) (2009) R100–10.
[135] M.J. Morris, T. Nguyen, Does neuropeptide Y contribute to the anorectic action of amylin? Peptides 22 (3) (2001) 541–546.
[136] X. Li, K. Fan, Q. Li, D. Pan, R. Hai, C. Du, Melanocortin 4 receptor-mediated effects of amylin on thermogenesis and regulation of food intake, Diabetes Metab. Res. Rev. 35 (5) (2019) e3149.
[137] Z. Su, A.L. Alhadeff, J.N. Betley, Nutritive, post-ingestive signals are the primary regulators of AgRP neuron activity, Cell Rep. 21 (10) (2017) 2724–2736.
[138] R.A. Essner, A.G. Smith, A.A. Jamnik, A.R. Ryba, Z.D. Trutner, M.E. Carter, AgRP neurons can increase food intake during conditions of appetite suppression and inhibit anorexigenic parabrachial neurons, J. Neurosci. 37 (36) (2017) 8678–8687.
[139] M.J. Krashes, S. Koda, C. Ye, S.C. Rogan, A.C. Adams, D.S. Cusher, E. Maratos-Flier, B.L. Roth, B.B. Lowell, Rapid, reversible activation of AgRP neurons drives feeding behavior in mice, J. Clin. Invest. 121 (4) (2011) 1424–1428.
[140] S. Luquet, F.A. Perez, T.S. Hnasko, R.D. Palmiter, NPY/AgRP neurons are essential for feeding in adult mice but can be ablated in neonates, Science 310 (5748) (2005) 683–685.
[141] C.A. Campos, A.J. Bowen, C.W. Roman, R.D. Palmiter, Encoding of danger by parabrachial CGRP neurons, Nature 555 (7698) (2018) 617–622.

[142] Y. Aponte, D. Atasoy, S.M. Sternson, AGRP neurons are sufficient to orchestrate feeding behavior rapidly and without training, Nat. Neurosci. 14 (3) (2011) 351–355.
[143] Q. Wu, M.P. Boyle, R.D. Palmiter, Loss of GABAergic signaling by AgRP neurons to the parabrachial nucleus leads to starvation, Cell 137 (7) (2009) 1225–1234.
[144] D. Atasoy, J.N. Betley, H.H. Su, S.M. Sternson, Deconstruction of a neural circuit for hunger, Nature 488 (7410) (2012) 172–177.
[145] C. Weyer, D.G. Maggs, A.A. Young, O.G. Kolterman, Amylin replacement with pramlintide as an adjunct to insulin therapy in type 1 and type 2 diabetes mellitus: a physiological approach toward improved metabolic control, Curr. Pharm. Des. 7 (14) (2001) 1353–1373.
[146] T.A. Lutz, J. Althaus, R. Rossi, E. Scharrer, Anorectic effect of amylin is not transmitted by capsaicin-sensitive nerve fibers, Am. J. Physiol. 274 (6 Pt 2) (1998) R1777–82.
[147] K.V. Andreassen, M. Feigh, S.T. Hjuler, S. Gydesen, J.E. Henriksen, H. Beck-Nielsen, C. Christiansen, M.A. Karsdal, K. Henriksen, A novel oral dual amylin and calcitonin receptor agonist (KBP-042) exerts antiobesity and antidiabetic effects in rats, American journal of physiology, Endocrinol. Metab. 307 (1) (2014) E24–33.
[148] R.D. Reidelberger, L. Kelsey, D. Heimann, Effects of amylin-related peptides on food intake, meal patterns, and gastric emptying in rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 282 (5) (2002) R1395–404.
[149] R.D. Reidelberger, U. Arnelo, L. Granqvist, J. Permert, Comparative effects of amylin and cholecystokinin on food intake and gastric emptying in rats, Am. J. Physiol. Regul. Integr. Comp. Physiol. 280 (3) (2001) R605–11.
[150] G.L. Edwards, B.R. Gedulin, C. Jodka, R.P. Dilts, C.C. Miller, A. Young, Area postrem (AP)-lesions block the regulation of gastric emptying by amylin, Neurogastroenterol. Motil. 10 (26) (1998).
[151] J. C, G. D, Y. A.A, G. B, GedulinAmylin modulation of gastric emptying in rats depends upon an intact vagus nerve (abstract), Diabetes 45 (1996) 235A.
[152] G.J. Ferrier, A.M. Pierson, P.M. Jones, S.R. Bloom, S.I. Girgis, S. Legon, Expression of the rat amylin (IAPP/DAP) gene, J. Mol. Endocrinol. 3 (1) (1989) R1–4.
[153] G.D. Bell, S. Reddy, X. Sun, Y. Yang, G.W. Krissansen, Distribution of insulin mRNA transcripts within the human body, Biochem. Biophys. Res. Commun. 451 (3) (2014) 425–430.
[154] H. Mulder, M. Ekelund, E. Ekblad, F. Sundler, Islet amyloid polypeptide in the gut and pancreas: localization, ontogeny and gut motility effects, Peptides 18 (6) (1997) 771–783.
[155] J.E. Koda, M. Fineman, T.J. Rink, G.E. Dailey, D.B. Muchmore, L.G. Linarelli, Amylin concentrations and glucose control, Lancet 339 (8802) (1992) 1179–1180.
[156] O.G. Kolterman, A. Gottlieb, C. Moyses, W. Colburn, Reduction of postprandial hyperglycemia in subjects with IDDM by intravenous infusion of AC137, a human amylin analogue, Diabetes Care 18 (8) (1995) 1179–1182.
[157] R.G. Thompson, A. Gottlieb, K. Organ, J. Koda, J. Kisicki, O.G. Kolterman, Pramlintide: a human amylin analogue reduced postprandial plasma glucose, insulin, and C-peptide concentrations in patients with type 2 diabetes, Diabetic Med. 14 (7) (1997) 547–555.
[158] W.A. Scherbaum, The role of amylin in the physiology of glycemic control, Exp. Clin. Endocrinol. Diabetes 106 (2) (1998) 97–102.
[159] J.H. Exton, Mechanisms of hormonal regulation of hepatic glucose metabolism, Diabetes. Metab. Rev. 3 (1) (1987) 163–183.
[160] R.G. Firth, P.M. Bell, H.M. Marsh, I. Hansen, R.A. Rizza, Postprandial hyperglycemia in patients with noninsulin-dependent diabetes mellitus. Role of hepatic and extrahepatic tissues, J. Clin. Invest. 77 (5) (1986) 1525–1532.
[161] G. Pehling, P. Tessari, J.E. Gerich, M.W. Haymond, F.J. Service, R.A. Rizza, Abnormal meal carbohydrate disposition in insulin-dependent diabetes. Relative contributions of endogenous glucose production and initial splanchnic uptake and effect of intensive insulin therapy, J. Clin. Invest. 74 (3) (1984) 985–991.
[162] A.T. Larsen, N. Sonne, K.V. Andreassen, M.A. Karsdal, K. Henriksen, Dose frequency optimization of the dual amylin and calcitonin receptor agonist KBP-088 –long-lasting improvement of food preference and body weight loss, J. Pharmacol. Exp. Ther. (2020).
[163] S. Gydesen, K.V. Andreassen, S.T. Hjuler, J.M. Christensen, M.A. Karsdal, K. Henriksen, KBP-088, a novel DACRA with prolonged receptor activation, is superior to davalintide in terms of efficacy on body weight, Am. J. Physiol. Endocrinol. Metab. 310 (10) (2016) E821-7.
[164] R.A. Silvestre, J. Rodriguez-Gallardo, C. Jodka, D.G. Parkes, R.A. Pittner, A.A. Young, J. Marco, Selective amylin inhibition of the glucagon response to arginine is extrinsic to the pancreas, Am. J. Physiol. Endocrinol. Metab. 280 (3) (2001) E443–9.
[165] A. Young, Role of amylin in nutrient intake - animal studies, Diabetic Med. 14 (Suppl 2) (1997) S14–8.